Ciproxifan, a histamine H₃ receptor antagonist, suppresses depolarization-evoked glutamate release in rat cerebrocortical nerve terminals via protein kinase A and mitogen-activated protein kinase signaling pathways

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INTRODUCTION

Histamine has long been studied for its inflammatory activity. Several recent studies have also demonstrated that H₃ receptor antagonists possess neuroprotective effect. However, the underlying mechanisms of the neuroprotective property of H₃ receptor antagonists have not been fully investigated. Given that glutamate plays a pivotal role in neuroprotection, the goal of this study was to examine the presynaptic effect of H₃ receptor antagonists on glutamate release and elucidate the underlying mechanisms.

MATERIAL AND METHODS

- ◆ Model: synaptosomes (truncated nerve terminals) purified from Sprague-Dawley rat cerebral cortex.
- ◆ Glutamate release was evoked by 4-aminopyridine (4-AP), a potassium channel blocker, which can destabilize the membrane potential and then cause repetitive Na⁺ channels firing that leads to the activation of voltage-dependent Ca²⁺ channels and neurotransmitter release.
- ◆ Glutamate was measured by enzyme-coupled on-line fluorimetry.
- ◆ The effect of ciproxifan, an H₃ antagonist, on glutamate release evoked by 4-AP was first examined.
- ◆ Membrane potential measurement: using positively charged membrane potential-sensitive dye, DiSC₃(5).
- ◆ Inhibitors of different Ca²⁺ channels were used to test which Ca²⁺ sources were affected by ciproxifan.
- ◆ Pharmacological activators and inhibitors of protein kinase cascades were used to investigate the signaling pathway.

RESULTS

Fig.1. (A) Ciproxifan inhibits 4-AP-evoked glutamate release from rat cerebrocortical nerve terminals (B) in a concentration-dependent manner. (***) $P < 0.001$.

Fig. 2. Blockade of Ciproxifan-mediated inhibition of 4-AP-evoked glutamate release by calcium chelation and a vesicular depletor, Bafilomycin A1, but not by the glutamate transporter inhibitor, DL-TBOA.

Fig. 3. Ciproxifan attenuates KCl-evoked glutamate release but fails to affect the synaptosomal membrane potential evoked by 4-AP.

Fig. 4. Suppression of ciproxifan-mediated inhibition of glutamate release by Cav2.1 and Cav2.2 channels blocker ω-conotoxin MVIIC but not by ryanodine receptor blocker dantrolene, or mitochondrial Na⁺/Ca²⁺ exchanger blocker CGP37157.

Fig. 5. Ciproxifan-mediated inhibition of 4-AP-induced glutamate release is prevented by PD98059, a MEK inhibitor, and H89, a PKA inhibitor.

CONCLUSIONS

- ◆ Our results suggest that ciproxifan inhibits glutamate release from rat cortical synaptosomes through the suppression of presynaptic voltage-dependent Ca²⁺ channels and both PKA and MAPK signaling cascades. These findings may delineate the possible neuroprotective mechanisms of H₃ antagonists.